Biochimica et Biophysica Acta 1843 (2014) 1457–1465

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Protein targeting via mRNA in bacteria ☆

Shanmugapriya Kannaiah, Orna Amster-Choder *

Department of Microbiology and Molecular Genetics, IMRIC, The Hebrew University – Faculty of Medicine, P.O.Box 12272, Jerusalem 91120, Israel

ARTICLE INFO

Article history:
Received 8 September 2013
Received in revised form 9 November 2013
Accepted 11 November 2013
Available online 19 November 2013

Keywords:
Protein targeting
Protein export
RNA localization
RNA zip-code
Subcellular organization
Bacterial cell

ABSTRACT

Proteins of all living organisms must reach their subcellular destination to sustain the cell structure and function. The proteins are transported to one of the cellular compartments, inserted into the membrane, or secreted across the membrane to the extracellular milieu. Cells have developed various mechanisms to transport proteins across membranes, among them localized translation. Evidence for targeting of Messenger RNA for the sake of translation of their respective protein products at specific subcellular sites in many eukaryotic model organisms have been accumulating in recent years. *Cis*-acting RNA localizing elements, termed RNA zip-codes, which are embedded within the mRNA sequence, are recognized by RNA-binding proteins, which in turn interact with motor proteins, thus coordinating the intracellular transport of the mRNA transcripts. Despite the rareness of conventional organelles, first and foremost a nucleus, pieces of evidence for mRNA localization to specific subcellular domains, where their protein products function, have also been obtained for prokaryotes. Although the underlying mechanisms for transcript localization in bacteria are yet to be unraveled, it is now obvious that intracellular localization of mRNA is a common mechanism to spatially localize proteins in both eukaryotes and prokaryotes. This article is part of a Special Issue entitled: Protein trafficking and secretion in bacteria. Guest Editors: Anastassios Economou and Ross Dalbey.

1. Introduction

Cells carry out a plethora of cellular processes to survive and compete in their ecological niche. In spite of these numerous processes, chaos does not seem to prevail in the cells. Rather, coherence is achieved by temporal and spatial organization of the various cellular events. A good example for such spatio-temporal coordination is the separation between synthesis of RNA molecules in the nucleus and their translation in the cytoplasm of eukaryotic cells. Recent studies of bacterial cells, previously regarded as non-compartmentalized, revealed complex cellular organization in these tiny cells, making it clear that many advanced mechanisms underlying cell architecture have evolved in these ancient organisms.

In eukaryotes, the majority of protein transport involves the endoplasmic reticulum (ER) and the Golgi apparatus. Once the synthesis of a secretory protein initiates, a hydrophobic N-terminal signal sequence, typically consisting of 16–30 amino acids, which emerges from the ribosome is recognized by the signal recognition particle (SRP) and is targeted along with the ribosome to the SRP receptors on the ER membrane in a process termed co-translational translocation. The elongating polypeptide chain then enters a channel called the Sec translocon for its translocation through the membrane. As the growing polypeptide moves into the ER lumen, the signal sequence is cleaved by a peptidase, so that the final secretory protein lacks the signal sequence. The entire translocation process is driven by ATP hydrolysis. Alternatively, secretion may also occur post-translationally, after the synthesis of the protein is completed. The post-translational secretion pathway is common in yeast. In the ER, the protein is properly folded and is transported in a vesicle to the cell surface via the Golgi apparatus, where the proteins undergo post-translational modifications. This pathway is called the classical secretory pathway, but many eukaryotic proteins are secreted by non-classical secretion pathways. Likewise, protein can be secreted from prokaryotic cells via two main routes, Sec-dependent, which shares many features with eukaryotic ER/Golgi secretion pathway, and Sec-independent [1–6].

2. mRNA targeting as a mechanism for localizing proteins

Undoubtedly, the signal peptide plays a significant role in protein translocation. However, its minimal length, although not strictly defined, is equivalent to 50 amino acids [7–9], which corresponds to the size of ribosome exit tunnel. This implies that the amino acid chain should be longer than 50 amino acids for a secretory protein. This has been shown not to be the case for many secretory proteins [10], plus many secreted proteins do not contain a recognizable signal sequence motif (see below). Hence, proteins encoded by small ORFs or those lacking a detectable signal sequence should use some other mechanism for their export.

Brunak and co-workers have listed many proteins that are found in the extracellular medium, which do not contain an apparent signal sequence, that is, are exported by none of the classical bacterial secretion

☆ This article is part of a Special Issue entitled: Protein trafficking and secretion in bacteria. Guest Editors: Anastassios Economou and Ross Dalbey.

* Corresponding author. Tel.: +972 2 675 8460; fax: +972 2 675 7910.

0167-4889/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbamcr.2013.11.004

pathways [6]. Most of the known examples come from the Gram-positive organism *Bacillus subtilis* [11]. Some of these proteins are also active in the cytoplasm, thus displaying a dual role, and are therefore called "moonlighting" proteins [12,13]. While attempting to identify a common motif that would represent this class of proteins, the authors came up with no simple secretion feature per se. Instead, they characterized them by a combination of sequence-derived features like arginine content, instability index [14] and amino acid composition, as well as structural features, such as secondary structure and transmembrane helices topology deduced by bioinformatics tools, to obtain a high-confidence prediction method. They show that secretory proteins in both Gram-positive and Gram-negative bacteria are generally more disordered than cytoplasmic proteins. They used their prediction method to verify reported information on proteins that are secreted via non-classical pathways and also applied it to identify other such proteins in *Escherichia coli* and *B. subtilis* via a global proteomic search. A prediction method, called SecretomeP, for the identification of mammalian secretory proteins, which are secreted through non-classical secretory pathways and are thus devoid of an N-terminal signal peptide, has also been developed [15].

Sorting of different proteins to their subcellular locations or to distinct organelles has been shown to involve mRNA localization in eukaryotes [1,16]. There are various potential advantages to localizing proteins to specific sites by targeting their mRNA transcripts: i) A cell would spend a lot of energy in translocating each protein molecule individually, whereas by localizing a transcript, which has the ability to undergo many rounds of protein synthesis, the energy spent by cells is expected to be significantly lower, ii) Targeting of mRNA transcripts helps in limiting the process of protein synthesis to the subcellular region that is close to where the proteins function. This helps the synthesized proteins to avoid non-specific interactions and protect them from being in a locality where they might exert or experience harmful effects, iii) Co-localization of mRNAs encoding different protein constituents of a complex facilitates complex formation, iv) Localization to specific subcellular domains might be advantageous for mRNAs whose translation needs to be delayed, and, v) Targeting may protect mRNAs from being exposed to ribonucleases and help in maintaining their proper level in the cell. Taken together, mRNA localization seems beneficial for regulating various cellular events and may provide a fitness benefit to the cells. In addition to transporting proteins with known transport route, RNA targeting is likely to play a role in transporting proteins whose route of transport is not known.

In recent years, *cis*-acting noncoding regions, termed RNA zip-codes, have been discovered to play a major role in the localization of RNA transcripts in eukaryotic cells [17]. The RNA zip-codes mostly reside in the 3′ untranslated region (3′ UTR), with some exceptions of localization elements that are present in the 5′ UTR or within the coding region (see below) [18–26]. Each zip-code element confers a certain localization pattern when added to any transcript [10,27,28]; removal of the zip-code abolishes this specific RNA localization pattern [28]. The zip-codes recruit RNA-binding proteins that are associated with motor proteins, which are anchored to the cytoskeleton, thus facilitating mRNA transport. Although the role of the UTRs in mRNA localization is well established, a common feature within them, which is shared by the zip-code elements, has not been established yet. Both structural [24,25,29–35] and sequence-based [36–38] features of the zip-codes are important for their identification.

Evidence suggesting a direct role for RNA in protein export was obtained by Rapoport and co-workers, who have shown that nuclear mRNA export can be mediated by a signal sequence coding region (SSCR) that can recruit factors for exporting secretory proteins-encoding mRNAs from the nucleus to the ER, independent of the canonical splicing-dependent pathway for nuclear export. The SSCR is hydrophobic in nature, a characteristic of signal sequences of secretory proteins that are usually located at the N-terminus of secreted proteins. The low content of adenine is another characteristic of the SSCR in all eukaryotes, although not in bacteria. The SSCR-mediated export pathway can act independently of the canonical pathway, but it can also be coupled to it for transporting mRNAs more efficiently [26].

Subsets of mRNAs, which share similar subcellular distribution or function, associate with certain RNA-binding proteins (RBP) that help them localize [39,40]. Accordingly, there are RBPs that associate specifically with mRNA encoding membrane or secretory proteins [40–42]. A well-studied example is the asymmetric localization of over twenty mRNAs to the yeast bud tip, the majority of which code for membrane and secretory proteins, which is mediated by the coordinated activity of Myo4p, She2p, and She3p [43–45]. This process involves recognition of the localized mRNA by the RNA-binding protein She2p and recruitment of Type V myosin, Myo4p, via the adaptor protein She3p [46,47]. Another example is the p180 RBP-mediated ER localization pathway, which has been shown to be required for efficient association of certain mRNAs with the ER in a ribosome-independent manner, i.e., independent of translation [48]. A lysine-rich region within p180 was shown to play a direct role by interacting with the mRNA transcripts that localize via this pathway. In another study, the Rrm4 RBP was shown to mediate localization of the mRNA encoding the secretory protein endochitinase Cts1; binding of Rrm4 to this mRNA is essential for efficient secretion of Cts1 in *Ustilago maydis* [49].

3. mRNA localization in prokaryotes

The architecture of eukaryotic cells, i.e., their organization into distinct compartments and the marked separation between the nucleus and cytoplasm, is considered to underlie the uncoupling between RNA synthesis and translation. Conversely, the prevailing view has been that transcription and translation are coupled in bacterial cells, as they largely lack membrane-engulfed organelles, primarily a nucleus. Recently, this dogma has been challenged in several studies. The development of advanced methodologies for observing and quantifying the dynamics of biomolecules, which enable visualization of the tiny bacterial cells, has made this progress possible. Various methods employed for visualizing mRNA have been discussed elsewhere [50,51]. The studies described below, which demonstrate the involvement of mRNA in protein subcellular localization and secretion in a translation-independent manner, support the emerging notion that bacteria are more complex than previously appreciated.

Besides fluorescence in situ hybridization (FISH), which has been used to observe RNA transcripts in fixed cell, a widely used approach for studying RNA localization in live eukaryotic cells is based on the use of the RNA-binding coat protein from MS2 bacteriophage (termed hereafter MS2 protein) fused to GFP and an mRNA tagged with tandem repeats of the MS2-binding site [52,53]. When expressed together, the MS2-GFP protein binds to its repeated binding sites on the RNA transcript and marks the RNA subcellular location. Golding and Cox adapted this system for bacteria to examine the movement of arbitrary RNA molecules in live *E. coli* cells, and were the first to highlight the intricacy of RNA distribution in bacterial cells [54]. Cluzel and coworkers used the MS2 system for single cell RNA profiling in bacterial cells. They showed that the diffusion time of the MS2-GFP fusion protein bound to mRNA is 30 times longer than unbound MS2-GFP, which facilitates the detection and visualization of bound MS2-GFP [55,56]. Subsequent studies, described below, used FISH and the MS2 system, as well as variations of these approaches, to visualize the distribution patterns of specific mRNAs in bacterial cells (Fig. 1 and Table 1) [57].

3.1. Cytoplasmic localization of mRNA transcripts

The regulation of the *lac* operon in *E. coli* has been extensively studied [58,59] and, hence, transcripts of its genes have been chosen time and again for visualization in different studies. One of the first studies of RNA localization monitored the localization of *lacZ* mRNA, which codes for the cytoplasmic β-D-galactosidase protein, using fluorescent

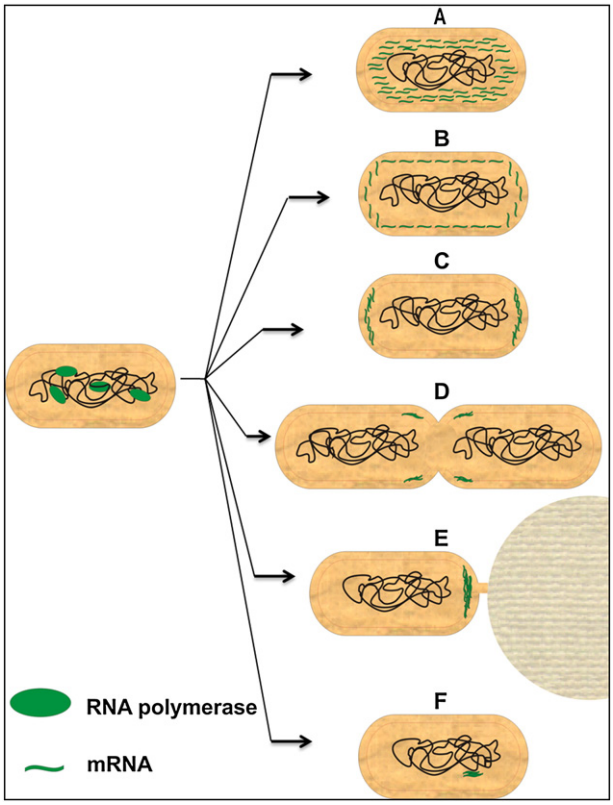

Fig. 1. Spatial distribution patterns of mRNA transcripts in bacteria. On the left: a bacterial cell showing RNA polymerase molecules transcribing the chromosome. On the right: (A–F): the different pattern of RNA localization observed thus far in bacterial cells. (A) Cytoplasmic localization: mRNA transcripts are distributed throughout the cytoplasm or in a helical pattern (not depicted) in the cytoplasm. (B) Membrane localization: mRNA transcripts are distributed around the cell circumference near the membrane. (C) Polar localization: mRNA transcripts localize near the cell poles. (D) Septal localization: mRNA transcripts localize to the septal regions during cell division. (E) Localization near sites of attachment to host cells: mRNA localization near the T3SS machinery allows injection of the translated T3SS proteins into the host cell. (F) Localization near sites of transcription: mRNA transcripts remain close to their transcription site on the chromosome, exhibiting limited dispersion in the cytoplasm.

protein complementation assay (PCA) with split EGFP (enhanced version of GFP) and the eIF4A RNA-binding protein. After analyzing the fluorescent signal by flow cytometry and fluorescence microscopy, *lacZ* mRNA was reported to be evenly distributed throughout the bacterial cell (Fig. 1A) [60].

Another RNA that codes for a well-known protein, whose subcellular localization has been studied in *E. coli* cells, is *cat* mRNA, which codes for chloramphenicol acetyl transferase, known to be a cytoplasmic protein [61]. The localization of *cat* mRNA was examined in live cells by the MS2 system and in fixed cells by FISH. Using both techniques, *cat* transcripts were observed in the cytoplasm (Fig. 1A), displaying a helix-like pattern [62]. The data obtained by the imaging techniques was further validated by separating the *E. coli* cells into membrane and cytosolic fractions and checking for the presence of *cat* mRNA in these fractions by reverse transcription followed by PCR or qPCR; *cat* transcripts were detected in the cytosolic fraction [62].

An additional *E. coli* gene, whose mRNA was detected in the cytoplasm by imaging and biochemical approaches, is *bglB* that codes for the cytoplasmic protein phospho-β-glucosidase (Fig. 1A). The *bglB* gene is part of the *bgl* operon [63], whose products are responsible for uptake and utilization of aromatic β-glucoside sugars [64,65]. The distribution pattern observed for the *bglB* mRNA transcripts is similar to the distribution of the *cat* transcripts, i.e., they spread out in the cytoplasm in a helix-like pattern [62]. The localization of transcripts of other genes of the *bgl* operon is discussed in the next sub-section.

We speculate that the distribution of cytoplasmic RNA in a helix-like pattern is due to the spatial organization of the *E. coli* nucleoid into a discrete, dynamic helical ellipsoid, as recently reported by Kleckner and co-workers [66]. The localization of the three mRNAs described above in the cytoplasm correlates with the subcellular localization of their encoded proteins. However, the question if this is the default distribution of non-localized mRNAs remains open. The localization of mRNAs at foci near their site of transcription in the cytoplasm, not necessarily correlating with localization of their protein products, is discussed in the Concluding remarks section.

3.2. Membrane, polar, and septal localization of mRNA transcripts

Different distribution patterns than the one observed for *lacZ*, *cat* and *bglB* mRNAs were observed for the mRNAs described below. First, the transcripts of the *lacY* gene that codes for the membrane-bound lactose permease that transports lactose and other galactosides into the cell [67], were shown to localize to the *E. coli* cell membrane by fluorescence microscopy and by cell fractionation followed by RT-PCR (Fig. 1B) [62]. Hence, the *lacZ* and *lacY* mRNAs, both transcribed from the *lac* operon, localize to the cytoplasm and to the membrane, respectively, in accordance with their respective protein products. It is worth mentioning that the polycistronic *lac* operon mRNA has been shown to be cleaved post-transcriptionally between the *lacY* and *lacZ* genes [68].

The *bglF* mRNA transcripts, which also encode a membrane-bound protein – the permease that transports β-glucosides into *E. coli* cells [63,69] – were detected at the cell membrane by imaging and biochemical approaches, similar to *lacY* transcripts (Fig. 1B) [62]. The *bglF* mRNA was shown to localize to the membrane also when its translation was inhibited in various ways, implying that the information for membrane localization is present within the transcript itself (RNA zip-code) [62]. In fact, the sequence encoding the membrane-spanning domain alone, and even the sequence encoding the first two transmembrane helices of this

Table 1
mRNA localization in bacteria.

mRNA	Organism	Site of localization	Function	Reference
bglB	*E. coli*	Cytoplasm	Hydrolyzes β-glucosides	[62]
bglF	*E. coli*	Membrane	Transports β-glucosides into the cell	[62]
bglG	*E. coli*	Poles	Antiterminator of *bgl* operon	[62]
cat	*E. coli*	cytoplasm	Confers resistance to chloramphenicol	[62]
comE	*B. subtilis*	Midcell septa	Involved in competence and development	[72]
creS	*C. crescentus*	Chromosomal site of transcription	Cell shape determination	[119]
divJ	*C. crescentus*	Chromosomal site of transcription	Regulation of cell division and differentiation	[119]
fliC	*E. coli*	Flagellar T3SS site on the membrane	A subunit of the flagellar filament	[97]
groESL	*C. crescentus*	Chromosomal site of transcription	Chaperone	[119]
lacY	*E. coli*	Membrane	Transports lactose and other galactosides into the cell	[62]
lacZ	*E. coli*	cytoplasm/chromosomal site of transcription	Cleaves lactose into glucose and galactose	[60,119]
ompA	*C. crescentus*	Chromosomal site of transcription	Diffusion channel	[119]
yopQ	*Y. enterocolitica*	T3SS site on the membrane	Required for virulence	[89]

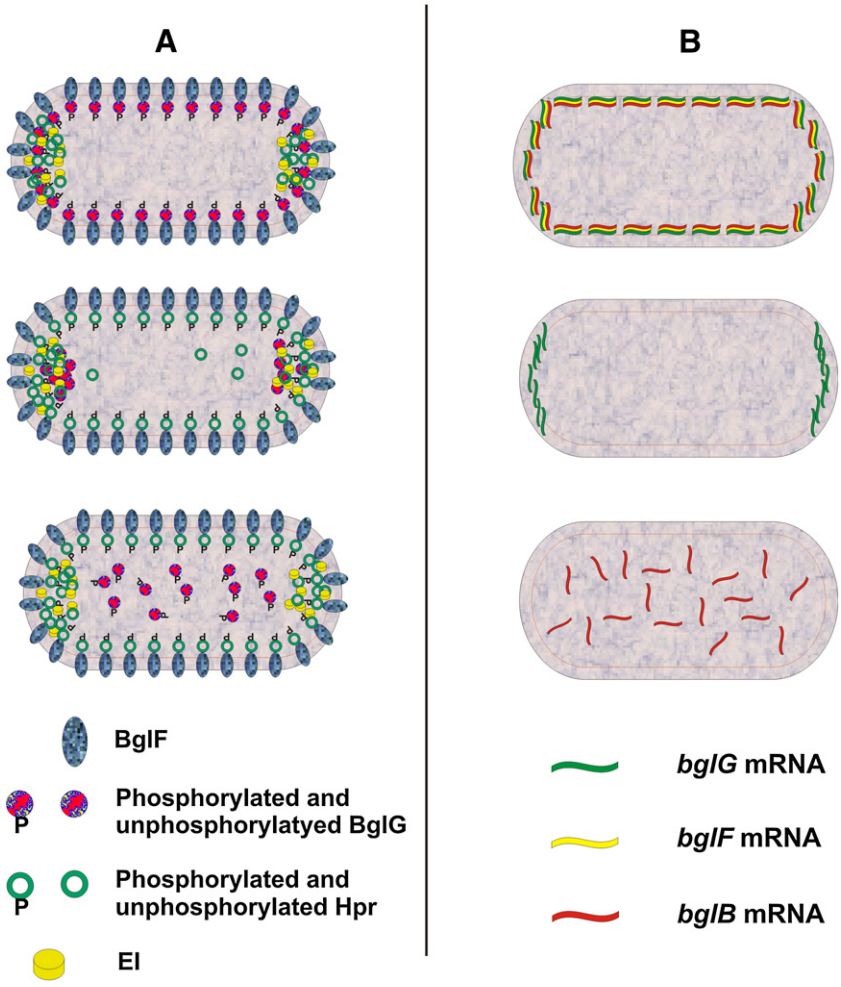

Fig. 2. Spatial organization of the components and mRNA transcripts of the PTS and Bgl sensory systems in *E. coli*. (A) Spatial organization of the Bgl and PTS proteins. Top: BglF is an integral membrane sugar permease, which forms a complex with the BglG transcription factor in the absence of the sugar. BglG is present in a phosphorylated and inactive state. The general PTS proteins, EI and HPr, are present at the cell poles. Middle: Upon the addition of sugar, BglG is dephosphorylated by BglF and migrates to the cell poles, where the PTS proteins are present. Also, HPr is phosphorylated by EI and is released to the cytoplasm and membrane, where it phosphorylates BglF, enabling it to transport the sugar into the cell. Bottom: BglG is activated by steric interactions with both EI and HPr and spreads throughout the cytoplasm. (B) Spatial organization of *bgl* transcripts. Top: The polycistronic *bglGFB* mRNA transcripts localize to the membrane. Middle: The monocistronic *bglG* mRNA transcripts localize to the poles. Bottom: The monocistronic *bglB* mRNA transcripts are distributed in a helical pattern (not depicted) in the cytoplasm. Together, the localization patterns illustrate the correlation between RNA localization and the requirement for complex formation, as well as the hierarchy in mRNA localization, i.e., the sequence encoding the membrane protein (BglF) is dominant in determining the subcellular localization of the mRNA.

domain, were shown to be sufficient for membrane-targeting of the mRNA. Moreover, the transcripts of other genes of the *bgl* operon, *bglB* and *bglG*, were detected in the membrane when they were a part of polycistronic transcripts that contain *bglF*, but demonstrated distinct localization patterns when expressed as monocistrons (Fig. 2B) [62]. Hence, a hierarchy exists, according to which the membrane-encoding sequence is dominant to the hydrophilic-encoding sequence in determining the localization of polycistronic transcripts [62].

Interestingly, although *bglG* mRNA that codes for a transcription factor, which regulates expression of the *bgl* operon, was detected at the cell membrane when expressed together with *bglF*, when expressed as a monocistron, the *bglG* transcripts were observed at the *E. coli* cell poles (Fig. 1C) [62]. Remarkably, similar localization patterns were observed for the BglG protein: in the absence of β-glucoside, BglG assembles with the BglF permease near the membrane to form a pre-complex that maintains BglG inactive [70]; in the presence of the sugar, BglG associates with the general proteins of the sugar phosphotransfer system (PTS) near the cell poles and is activated by them (Fig. 2A) [71]. Therefore, the localization patterns of the *bglG* transcripts correlate with the requirements for complex formation.

An intriguing result was obtained when a gene encoding a *Drosophila melanogaster* transmembrane protein was expressed in *E. coli*: the transcripts of this protein localize to bacterial cell membrane, suggesting that at least some membrane-targeting signals may have been conserved across the eukaryotic–prokaryotic divide.

Yet another pattern of mRNA localization was reported by Gueiros-Filho and co-workers, who studied post-transcriptional control of the late competence operon *comE* by the ComN protein in *B. subtilis*. The authors used the MS2 system to show that ComN, together with DivIVA promotes the localization of *comE* mRNA to the midcell septa and the poles (Fig. 1D) [72]. Interestingly, the DivIVA protein, which is involved in the differentiation of the *B. subtilis* cell poles, localizes first to midcell, which is the future division site, and then to the poles, whereas the ComN protein localizes to the division site and cell poles in a DivIVA-dependent manner. The localization of the *comE* mRNA is impaired in a *comN* mutant and a *divIVA min* mutant. The authors speculate that localization of the *comE* mRNA favors the accumulation of ComEC, which constitutes the DNA transport pore [73], at the poles, thus supporting competence and development.

In eukaryotes, the localization of mRNA to the ER has been associated with global protein synthesis [74]. It is also suggested to be involved in the synthesis of proteins necessary for cell division and equal distribution of biomolecules attached to the partitioning ER during cytokinesis [75]. Likewise, it is possible that mRNA localization to mid-septum and

poles, preferably the pole derived from the parent cell that becomes the old pole in the daughter cell, might be involved in the synthesis of cell division proteins and equal distribution of proteins in bacteria during cytoplasm partitioning into two daughter cells. Additional studies are required to shed light on the role of mRNA localization in localized translation in bacteria.

A mechanism by which active ribosomes and RNA transcripts that encode integral membrane proteins, are targeted to the membrane in an SRP-independent manner has recently been proposed by Bibi [76]. According to this model, the SRP receptor, FtsY might mediate localization of the ribosomes to the membranes; transcripts encoding membrane proteins localize to the membrane by an inherent property of the transcripts and associate with the ribosomes, which already reside at the membrane; the hydrophobic polypeptide that emerges as a result of membrane protein synthesis is recognized by the SRP and then the membrane protein assembles on the Sec translocon. Bibi suggests that this model would explain the finding that SRP depletion affects the assembly of membrane proteins, but not their targeting or expression. This model assigns a direct role to the RNA transcripts in the insertion of proteins to the inner membrane, albeit this hypothetic model still needs to be confirmed.

3.3. Localization of mRNA transcripts encoding secretory proteins

Bacteria can be broadly classified into Gram-positive and Gram-negative bacterial species, the first group has a thicker peptidoglycan layer, but only a single membrane, whereas the second group has an additional outer membrane, which makes the secretion process topologically more complex [77,78]. To facilitate protein export across this barrier, Gram-negative bacteria have evolved numerous pathways for protein secretion, which can be categorized into nine major groups: (i) type I secretion system (T1SS); (ii) type II secretion system (T2SS); (iii) type III secretion system (T3SS); (iv) type IV secretion system (T4SS); (v) type V secretion system (T5SS) (also called the autotransporter [AT]); (vi) type VI secretion system (T6SS); (vii) Twin-Arginine translocation system (TAT); (viii) YidC insertase and; (ix) chaperone/usher (CU) secretion system [79]. Some of these pathways are Sec-dependent. i.e., they require the Sec translocase, which consists of the SecYEG protein-conducting channel, the SecB chaperone and the SecA motor protein, to cross the inner membrane. Secretory proteins destined to reside in the periplasm or in the outer membrane are predominantly targeted to the Sec translocase post-translationally, by virtue of their signal sequence that is recognized by SecB or SecA, whereas inner membrane proteins are targeted co-translationally as ribosome-bound nascent chains in an SRP-dependent manner [80,81]. Other pathways – T1SS, T3SS, T5SS, T6SS, TAT and Yid C insertase – are Sec-independent; the first four systems translocate proteins directly from the cytoplasm to the extracellular environment or to a host cell, whereas the TAT system translocates folded proteins across the inner membrane exclusively [82] and the YidC insertase integrates membrane proteins into the cytoplasmic membrane and can also act in cooperation with the Sec translocase [83]. Secreted proteins in Gram-positive bacteria usually possess a signal sequence and are transported across the single membrane mostly through the Sec pathway. The proteins are either processed during their translocation or shortly thereafter and remain anchored to the peptidoglycan cell wall via the sortase group of proteins or are released to the extracellular milieu [84].

Is mRNA targeting also involved in localizing secretory proteins? Examples for such an involvement in T3SS-mediated secretion are briefly discussed below. T3SS directly translocates pathogenicity effector proteins from the bacterial cell into the cytosol of the eukaryotic host cell [85]. Although this system has been extensively studied, and despite the availability of methods for predicting proteins that are transported via T3SS [86], the exact nature of the T3SS signal sequence remains elusive and controversial. Suggestions for the nature of the signal for secretion via the T3SS range from increased structural flexibility of the coiled-coil domains, which are typical to T3SS proteins [87], to certain features of the mRNA sequence and of the amino acid sequence of the T3SS proteins.

The proteins that seem to best represent secretion by T3SS are the Yop proteins in pathogenic *Yersinia* species [88]. The expression of the Yop proteins can be induced either by contact with eukaryotic cells or by growing the bacteria in a calcium deficient medium at an elevated temperature. Once expressed, the Yop proteins are secreted into the eukaryotic cell via the T3SS, leading to its death. No obvious amino acid sequence that could direct the Yop proteins to the T3SS has been identified. Anderson and Schneewind studied secretion of the virulent protein YopQ in the *Yersinia enterocolitica* strain W22703 [89]. YopQ is not detected in the cytoplasm, it lacks an apparent secretion signal, and the 3′ UTR of its encoding mRNA is dispensable for secretion. The authors identified an mRNA signal, contained within the first 15 codons of *yopQ* open reading frame, as responsible for YopQ secretion (Fig. 1E). They then went further to show that YopQ polypeptide, synthesized prior to mRNA targeting, could not be secreted, thus highlighting the mRNA signal as the sole element responsible for YopQ secretion [89].

Besides YopQ, other Yop proteins, including YopE and YopN, were suggested to use an RNA element for their secretion. Importantly, frameshift mutations in this putative element sequence, which altered the amino acid sequence encoded by it, had little effect on secretion, whereas silent mutations at the wobble base positions, which altered the RNA structure, dramatically affected secretion. In fact, such mutations completely blocked secretion, thus indicating an important role for the mRNA sequence in Yop protein secretion [89–93]. On the other hand, YopQ in other serotypes of *Y. enterocolitica*, as well as the YopQ homologue in *Yersinia pseudotuberculosis* (called YopK) accumulate intracellularly [94,95], suggesting that the mRNA signal hypothesis might not apply to all *Yersinia* species.

mRNA was also suggested to play an important role in secretion by the flagellar T3SS, a variant of T3SS that secretes structural and regulatory components of the flagellum, including the flagellin protein (FliC), which constitutes the filament of the bacterial flagellum and is one of the most abundant proteins in the cell [96]. The flagellar T3SS secretes both flagellar and non-flagellar proteins and it has been shown to use an mRNA signal for this process. Hence, when the 173 bp untranslated region of *fliC* was introduced upstream of various genes, it efficiently directed secretion of their protein products into the growth medium [97].

A similar approach was applied to examine the role of untranslated regions of mRNAs that encode T3SS proteins, which are involved in *Salmonella* virulence, in injection of proteins to macrophages. The 3′-UTR of forty two mRNAs encoding *Salmonella* T3SS effectors, each 25 bp in length, was fused to *cya*, encoding the adenylate cyclase reporter protein. Secretion was evaluated by means of cyclic AMP (cAMP) levels in the macrophage [98]. The 3′-UTR sequence of five effectors, *gtgA*, *cigR*, *gogB*, *sseL*, and *steD*, efficiently translocated adenylate cyclase into macrophages. A minimal element of 7 bp was sufficient for the translocation. The secretion-promoting 3′-UTR sequences did not possess any consensus motif or a particular secondary structure, but the 3′-UTRs of three of the five effectors, *gtgA*, *gogB*, and *sseL* were shown to act via the RNA chaperone Hfq, as mutations in *hfq* abolished translocation of the respective fusion proteins. The Hfq was shown to stabilize the RNA transcripts and the translation of full-length proteins, suggesting that a complex between the RNA signal and Hfq participates in the secretion process. Still, when the 3′-UTRs of *gtgA* was fused to the gene encoding enolase, the protein was not injected into the host cells, suggesting that the identified sequence is not a general motif for protein secretion, but is rather specific for certain proteins [98].

Communication via mRNAs that are secreted from eukaryotic cells in membrane vesicles has been documented [99]. Bacteria produce membrane vesicles during infections, and these vesicles are thought to establish a communication with the host [100,101]. For example, the *E. coli* pore-forming protein ClyA, which exerts cytotoxic effects on the

host cells, is released from *E. coli* cells and delivered to mammalian cells via outer-membrane vesicles [102]. We thus hypothesize that, in analogy to eukaryotes, the outer-membrane vesicles produced by bacteria may also serve as non-conventional means for secreting mRNA transcripts into the extracellular milieu and/or delivering bacterial mRNAs to host cells.

3.4. RNA zip-codes

RNA zip-codes, which are *cis*-acting elements that play an essential role in the subcellular localization of RNA transcripts, have been identified and characterized in eukaryotic cells [17]. Most of these RNA targeting signals are located in untranslated regions of the mRNA transcripts, with some exceptions. In prokaryotes, the organization of genes in polycistronic operons with relatively short untranslated regions [103] suggests that bacterial RNA zip-codes might be located within open reading frames. Indeed, *cis*-acting sequences within the transmembrane-coding sequence of the BglF protein, were shown to be necessary and sufficient for targeting the *bgl* operon transcripts to the membrane [62].

Prilusky and Bibi applied a bioinformatic approach to identify the features that specify RNA localization elements within bacterial open reading frames [104]. The identified feature revealed a strong bias for uracil-rich codons in the mRNA of integral membrane proteins. Hence, the codons for very hydrophobic amino acids that are highly represented in regions encoding membrane-spanning domains contain approximately 50% uracils. Significantly, the U-richness trait is not confined to hydrophobic amino acid, since hydrophilic amino acids that are overrepresented in integral membrane proteins, like serine and threonine, are also encoded by U-rich codons. The uracil bias is conserved also in mRNAs of eukaryotic membrane proteins, but to a lesser extent, suggesting that this preference declined through evolution [104].

Studies aimed at deciphering and characterizing mRNA localization elements have only begun in prokaryotes. Of note, this is a relatively young research field also in eukaryotes. The emerging view from studies of eukaryotic mRNA localization elements is that they involve a combination of sequence and structure [105,106]. Although in a few cases primary sequence motifs for RNA localization were identified, in general, localization signals seem to operate at the level of secondary and tertiary structure of the transcripts [106]. As the number of mRNAs displaying specific localization increases, the nature of the RNA zip-codes is expected to unravel.

3.5. Localization of membrane protein-encoding mRNAs by transertion

An additional mechanism that brings mRNA of integral membrane proteins to the bacterial cell membrane is transertion. This mechanism couples transcription/translation of membrane protein-encoding genes to insertion of their protein products into the membrane. The physical association of the chromosome with the membrane is thought to be mediated by the emerging nascent mRNA transcript. Although a direct proof that transertion operates in live cells is hard to obtain, indirect proofs suggested that this phenomenon accounts for certain aspects of cell physiology, mainly membrane heterogeneity and chromosome segregation [107,108]. Recently, Goulian and co-workers provided direct evidence for the repositioning of chromosomal genetic loci, which express integral membrane proteins, to the vicinity of the membrane upon the expression of these genes in *E. coli* cells [109]. The non-homogenous distribution of phospholipids in the bacterial cytoplasmic membrane [110] might act as a cue to trigger the mRNA and mRNA-mediated chromosome relocation towards the membrane.

3.6. Localization of RNA fate-determining complexes: ribosomes and RNA degradosome

The spatial distribution of ribosomes and RNA polymerase in bacterial cells has been investigated by several groups. Errington and co-workers have shown that RNA polymerase resides primarily within the region of the nucleoid, whereas the ribosomes occupy the region outside the nucleoid in *B. subtilis* cells (Fig. 3A) [111]. Zhuang and co-workers used superresolution microscopy to study the distribution of major nucleoid-associated proteins (NAPs) in *E. coli* cells [112]. Four NAPs – HU, Fis, IHF, and StpA – were observed as scattered throughout the nucleoid, whereas a fifth one – HNS, a global transcription silencer – formed few compact clusters per chromosome. On the other hand, the ribosomes were enriched in the cell periphery, well secluded from the nucleoid. This is in agreement with the results documented by Weisshaar and co-workers, who have also observed a strong segregation of the nucleus and the associated RNA polymerase molecules from the ribosomes in *E. coli* cells using superresolution microscopy [113]. Taken together, these studies suggest that at least a fraction of the RNA transcripts in *B. subtilis* and *E. coli* cells move away from the nucleus to other subcellular domains for their translation (Fig. 3A).

A correlation between the localization of the RNA transcripts and of the RNA degradation machinery is also expected to exist. RNase E, a major component of the mRNA degradosome, was reported to follow

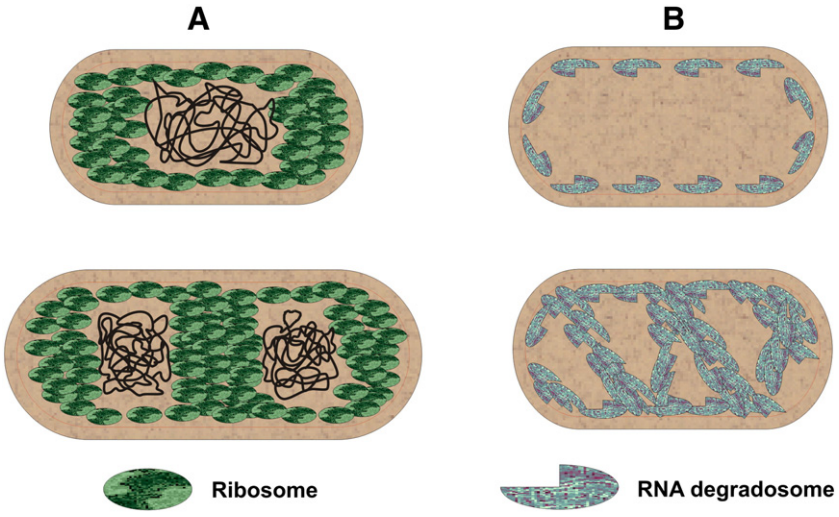

Fig. 3. Spatial distribution of the translation and RNA degradation machineries in *E. coli*. (A) Top: Ribosomes are enriched near the periphery of the cell distinct from chromosome, that is, at the cell poles and membrane. Bottom: In a dividing cell, the ribosomes are present also at the septal regions in midcell, in addition to the poles and membrane. (B) Components of the RNA degradation machinery of *E. coli*, called RNA degradosome, were detected at the cell membrane (top) and in a helical pattern (bottom).

a helical structure and to associate with membrane in *E. coli* (Fig. 3B) [114–116]. Other components of the degradosome, RhlB, PNPase, and enolase, which associate with RNase E to form the RNA decay complex, are also organized in a similar fashion [116]. Similarly, RNase Y of *B. subtilis* was also found to be membrane-associated [117,118]. The relative distribution patterns of the RNA transcripts and the degradation machinery can either limit the interaction of certain mRNAs with the degradosome or provide a mechanism to subject mRNAs to decay, to guarantee proper levels of mRNAs in the cell and, thus, of proteins.

Of note, the ribosomes and RNase E in *Caulobacter crescentus* cells were reported to have different localization patterns than those documented in *E. coli* and *B. subtilis* cells, as both machineries co-localize with the *C. crescentus* nucleoid [119].

Bacterial small RNAs (sRNAs) regulate gene expression at post-transcriptional levels, usually by affecting stability or translation of specific mRNAs via annealing of the sRNAs with these mRNA targets [120]. An example is provided by *sgrS*, an sRNA that inhibits translation of *ptsG* mRNA and targets it to RNaseE for degradation [121,122]. Similarly, there are sRNAs that positively regulate their mRNA targets by stabilizing them (e.g., the sRNA *gadY* upregulates the level of *gadX* mRNA, [123]). sRNAs act in conjunction with Hfq, an RNA chaperone. Electron microscopy studies have shown that Hfq concentrates in the vicinity of the cytoplasmic membrane [124,125]. It is expected that the relative spatial organization of the sRNAs, their mRNA targets, the Hfq protein and the degradosome coordinates the fate of the mRNAs.

4. Concluding remarks

Due to the lack of well-defined intracellular organelles in bacterial cells, they were considered inappropriate model organisms for studying cellular architecture. The widely held view that prevailed for many years has been that transcription and translation are coupled in bacteria, that is, the nascent mRNA transcript that emerges from the RNA polymerase is captured directly by ribosomes for protein synthesis. This view implies that the nucleoid, the RNA polymerase and the ribosomes are all located in the same vicinity. The advancement in imaging technologies has helped to obtain a blueprint of bacterial cell organization, which is different than expected, according to which only 10–15% of the ribosomes lie within the nucleoid lobes [113]. These findings challenge the transcription–translation coupling model, or at least the extent to which it holds true. The view that is currently developing is that bacterial cells have intricate cellular organization and functional compartmentalization. Thus, the notion that, like in eukaryotes, each step in bacterial gene expression is spatially regulated is becoming increasingly accepted. Still, the option that translation begins during transcription with 10–15% of the ribosome population and then the mRNA moves outside or to the edge of the nucleoid cannot be ruled out. Independent of the degree of coupling between transcription and translation, the idea that RNA localization, either in combination with or independent of translation, may be involved in localizing proteins to different subcellular domains or in secreting them outside the cell, is novel to the field.

Not all studies of RNA localization carried out thus far support a role for RNA targeting in protein localization. Jacobs-Wagner and co-workers detected various mRNAs in *C. crescentus* cells, as well as one mRNA in *E. coli*, close to their site of transcription on the chromosome, with limited dispersion in the cytoplasm (Fig. 1F) [119]. Notably, the mRNA monitored in *E. coli*, *lacZ*, has previously been shown to spread uniformly in the cytoplasm by Broude and co-workers (see above; [60]). This difference in the observed dispersion of the same mRNA might be due to the use of different RNA imaging methodologies or to the differences in the expression system. The RNA localization pattern in *C. crescentus* cells significantly coincides with the spatial organization of the actively translating ribosomes and the RNase E in these cells [119], which in *E. coli* cells localize mainly outside the nucleoid lobes and near the membrane [113,114]. Notably, Campos and Jacobs-Wagner have recently suggested that spatial organization of mRNA-related processes is different in *C. crescentus* and in *E. coli* cells, correlating with the different distribution of the ribosomes and of the mRNA decay machinery in the two organisms [126].

The fact that bacterial mRNAs are present in low copy number and have short half-life, not exceeding few minutes [127], challenges our ability to study mRNA localization in these cells. However, using new methodologies and reagents for cell imaging, mRNAs in bacterial cells were recently shown to localize to distinct subcellular domains — poles, membrane, cytoplasm, septum and chromosomal genetic loci where their protein products are synthesized (Table 1). Indirect evidence, such as the spatial distribution of the bacterial RNA degradosome, suggests that beside localized translation, RNA localization might play a role in processes like mRNA degradation.

How are mRNA transcripts targeted? What factors dictate their localization? Is it an active or a passive process? Are cytoskeletal elements involved in this process, similar to their role in eukaryotic cells? These are few basic questions that need to be answered by future studies. Nonetheless, it is evident that mRNA localization plays a major role in keeping the major post-transcriptional processes, like transcription, translation and RNA degradation, under spatial constraints and in linking and coordinating the processes of transcription, translation, and protein export.

Acknowledgements

We thank all members of the Amster-Choder lab for fruitful discussions. This work was supported by the Israel Science Foundation founded by the Israel Academy of Sciences and Humanities.

References

[1] B.C. Cross, I. Sinning, J. Luirink, S. High, Delivering proteins for export from the cytosol, Nat. Rev. Mol. Cell Biol. 10 (2009) 255–264.
[2] C.K. Barlowe, E.A. Miller, Secretory protein biogenesis and traffic in the early secretory pathway, Genetics 193 (2013) 383–410.
[3] Z. Cheng, Protein translocation through the Sec61/SecY channel, Biosci. Rep. 30 (2010) 201–207.
[4] T.A. Rapoport, Protein translocation across the eukaryotic endoplasmic reticulum and bacterial plasma membranes, Nature 450 (2007) 663–669.
[5] T.A. Rapoport, Protein transport across the endoplasmic reticulum membrane, FEBS J. (2008) 4471–4478(England).
[6] J.D. Bendtsen, L. Kiemer, A. Fausboll, S. Brunak, Non-classical protein secretion in bacteria, BMC Microbiol. 5 (2005) 58.
[7] I.M. Ibrahimi, D. Cutler, D. Stueber, H. Bujard, Determinants for protein translocation across mammalian endoplasmic reticulum. Membrane insertion of truncated and full-length prelysozyme molecules, Eur. J. Biochem. 155 (1986) 571–576.
[8] V. Siegel, P. Walter, Functional dissection of the signal recognition particle, Trends Biochem. Sci. 13 (1988) 314–316.
[9] B. Jungnickel, T.A. Rapoport, A posttargeting signal sequence recognition event in the endoplasmic reticulum membrane, Cell 82 (1995) 261–270.
[10] A. Loya, L. Pnueli, Y. Yosefzon, Y. Wexler, M. Ziv-Ukelson, Y. Arava, The 3′-UTR mediates the cellular localization of an mRNA encoding a short plasma membrane protein, RNA 14 (2008) 1352–1365.
[11] H. Tjalsma, H. Antelmann, J.D. Jongbloed, P.G. Braun, E. Darmon, R. Dorenbos, J.Y. Dubois, H. Westers, G. Zanen, W.J. Quax, O.P. Kuipers, S. Bron, M. Hecker, J.M. van Dijl, Proteomics of protein secretion by *Bacillus subtilis*: separating the "secrets" of the secretome, Microbiol. Mol. Biol. Rev. 68 (2004) 207–233.
[12] J. Schaumburg, O. Diekmann, P. Hagendorff, S. Bergmann, M. Rohde, S. Hammerschmidt, L. Jansch, J. Wehland, U. Karst, The cell wall subproteome of *Listeria monocytogenes*, Proteomics 4 (2004) 2991–3006.
[13] C.J. Jeffery, Moonlighting proteins, Trends Biochem. Sci. 24 (1999) 8–11.
[14] K. Guruprasad, B.V. Reddy, M.W. Pandit, Correlation between stability of a protein and its dipeptide composition: a novel approach for predicting in vivo stability of a protein from its primary sequence, Protein Eng. 4 (1990) 155–161.
[15] J.D. Bendtsen, L.J. Jensen, N. Blom, G. Von Heijne, S. Brunak, Feature-based prediction of non-classical and leaderless protein secretion, Protein Eng. Des. Sel. 17 (2004) 349–356.
[16] A. Bashirullah, R.L. Cooperstock, H.D. Lipshitz, RNA localization in development, Annu. Rev. Biochem. 67 (1998) 335–394.
[17] H. Chabanon, I. Mickleburgh, J. Hesketh, Zipcodes and postage stamps: mRNA localisation signals and their trans-acting binding proteins, Brief. Funct. Genomic Proteomic 3 (2004) 240–256.
[18] Y. Oleynikov, R.H. Singer, RNA localization: different zipcodes, same postman? Trends Cell Biol. 8 (1998) 381–383.

[19] J.E. Hesketh, Sorting of messenger RNAs in the cytoplasm: mRNA localization and the cytoskeleton, Exp. Cell Res. 225 (1996) 219–236.
[20] R.P. Jansen, mRNA localization: message on the move, Nat. Rev. Mol. Cell Biol. 2 (2001) 247–256.
[21] C. Saunders, R.S. Cohen, The role of oocyte transcription, the 5′UTR, and translation repression and derepression in *Drosophila gurken* mRNA and protein localization, Mol. Cell 3 (1999) 43–54.
[22] G.L. Thio, R.P. Ray, G. Barcelo, T. Schupbach, Localization of *gurken* RNA in *Drosophila* oogenesis requires elements in the 5′ and 3′ regions of the transcript, Dev. Biol. 221 (2000) 435–446.
[23] M. Capri, M.J. Santoni, M. Thomas-Delaage, O. Ait-Ahmed, Implication of a 5′ coding sequence in targeting maternal mRNA to the *Drosophila* oocyte, Mech. Dev. 68 (1997) 91–100.
[24] P. Chartrand, X.H. Meng, R.H. Singer, R.M. Long, Structural elements required for the localization of ASH1 mRNA and of a green fluorescent protein reporter particle in vivo, Curr. Biol. 9 (1999) 333–336.
[25] I. Gonzalez, S.B. Buonomo, K. Nasmyth, U. von Ahsen, ASH1 mRNA localization in yeast involves multiple secondary structural elements and Ash1 protein translation, Curr. Biol. 9 (1999) 337–340.
[26] A.F. Palazzo, M. Springer, Y. Shibata, C.S. Lee, A.P. Dias, T.A. Rapoport, The signal sequence coding region promotes nuclear export of mRNA, PLoS Biol. 5 (2007) e322.
[27] E.H. Kislauskis, Z. Li, R.H. Singer, K.L. Taneja, Isoform-specific 3′-untranslated sequences sort alpha-cardiac and beta-cytoplasmic actin messenger RNAs to different cytoplasmic compartments, J. Cell Biol. 123 (1993) 165–172.
[28] J.L. Veyrune, G.P. Campbell, J. Wiseman, J.M. Blanchard, J.E. Hesketh, A localisation signal in the 3′ untranslated region of c-myc mRNA targets c-myc mRNA and beta-globin reporter sequences to the perinuclear cytoplasm and cytoskeletal-bound polysomes, J. Cell Sci. 109 (Pt 6) (1996) 1185–1194.
[29] G. Bermano, R.K. Shepherd, Z.E. Zehner, J.E. Hesketh, Perinuclear mRNA localisation by vimentin 3′-untranslated region requires a 100 nucleotide sequence and intermediate filaments, FEBS Lett. 497 (2001) 77–81.
[30] P.M. Macdonald, K. Kerr, J.L. Smith, A. Leask, RNA regulatory element BLE1 directs the early steps of bicoid mRNA localization, Development 118 (1993) 1233–1243.
[31] D. Ferrandon, L. Elphick, C. Nusslein-Volhard, D. St Johnston, Staufen protein associates with the 3′UTR of bicoid mRNA to form particles that move in a microtubule-dependent manner, Cell 79 (1994) 1221–1232.
[32] P.M. Macdonald, K. Kerr, Mutational analysis of an RNA recognition element that mediates localization of bicoid mRNA, Mol. Cell. Biol. 18 (1998) 3788–3795.
[33] C. Wagner, I. Palacios, L. Jaeger, D. St Johnston, B. Ehresmann, C. Ehresmann, C. Brunel, Dimerization of the 3′UTR of bicoid mRNA involves a two-step mechanism, J. Mol. Biol. 313 (2001) 511–524.
[34] T.L. Serano, R.S. Cohen, A small predicted stem-loop structure mediates oocyte localization of Drosophila K10 mRNA, Development 121 (1995) 3809–3818.
[35] S.L. Bullock, D. Zicha, D. Ish-Horowicz, The Drosophila hairy RNA localization signal modulates the kinetics of cytoplasmic mRNA transport, EMBO J. 22 (2003) 2484–2494.
[36] K. Ainger, D. Avossa, A.S. Diana, C. Barry, E. Barbarese, J.H. Carson, Transport and localization elements in myelin basic protein mRNA, J. Cell Biol. 138 (1997) 1077–1087.
[37] E.H. Kislauskis, X. Zhu, R.H. Singer, Sequences responsible for intracellular localization of beta-actin messenger RNA also affect cell phenotype, J. Cell Biol. 127 (1994) 441–451.
[38] T.P. Munro, R.J. Magee, G.J. Kidd, J.H. Carson, E. Barbarese, L.M. Smith, R. Smith, Mutational analysis of a heterogeneous nuclear ribonucleoprotein A2 response element for RNA trafficking, J. Biol. Chem. 274 (1999) 34389–34395.
[39] A.P. Gerber, D. Herschlag, P.O. Brown, Extensive association of functionally and cytotopically related mRNAs with Puf family RNA-binding proteins in yeast, PLoS Biol. 2 (2004) E79.
[40] D.J. Hogan, D.P. Riordan, A.P. Gerber, D. Herschlag, P.O. Brown, Diverse RNA-binding proteins interact with functionally related sets of RNAs, suggesting an extensive regulatory system, PLoS Biol. 6 (2008) e255.
[41] A. Jambhekar, K. McDermott, K. Sorber, K.A. Shepard, R.D. Vale, P.A. Takizawa, J.L. DeRisi, Unbiased selection of localization elements reveals cis-acting determinants of mRNA bud localization in Saccharomyces cerevisiae, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 18005–18010.
[42] N. Colomina, F. Ferrezuelo, H. Wang, M. Aldea, E. Gari, Whi3, a developmental regulator of budding yeast, binds a large set of mRNAs functionally related to the endoplasmic reticulum, J. Biol. Chem. 283 (2008) 28670–28679.
[43] M. Schmid, A. Jaedicke, T.G. Du, R.P. Jansen, Coordination of endoplasmic reticulum and mRNA localization to the yeast bud, Curr. Biol. 16 (2006) 1538–1543.
[44] S. Aronov, R. Gelin-Licht, G. Zipor, L. Haim, E. Safran, J.E. Gerst, mRNAs encoding polarity and exocytosis factors are cotransported with the cortical endoplasmic reticulum to the incipient bud in *Saccharomyces cerevisiae*, Mol. Cell. Biol. 27 (2007) 3441–3455.
[45] C. Genz, J. Fundakowski, O. Hermesh, M. Schmid, R.P. Jansen, Association of the yeast RNA binding protein She2p with tubular endoplasmic reticulum depends on membrane curvature, J. Biol. Chem. 288 (2013) 32384–32393.
[46] R.M. Long, W. Gu, E. Lorimer, R.H. Singer, P. Chartrand, She2p is a novel RNA-binding protein that recruits the Myo4p-She3p complex to ASH1 mRNA, EMBO J. 19 (2000) 6592–6601.
[47] F. Bohl, C. Kruse, A. Frank, D. Ferring, R.P. Jansen, She2p, a novel RNA-binding protein tethers ASH1 mRNA to the Myo4p myosin motor via She3p, EMBO J. 19 (2000) 5514–5524.
[48] X.A. Cui, H. Zhang, A.F. Palazzo, p180 promotes the ribosome-independent localization of a subset of mRNA to the endoplasmic reticulum, PLoS Biol. 10 (2012) e1001336.
[49] J. Koepke, F. Kaffarnik, C. Haag, K. Zarnack, N.M. Luscombe, J. Konig, J. Ule, R. Kellner, D. Begerow, M. Feldbrugge, The RNA-binding protein Rrm4 is essential for efficient secretion of endochitinase Cts1, Mol. Cell. Proteomics 10 (2011)(M111.011213).
[50] T.T. Weil, R.M. Parton, I. Davis, Making the message clear: visualizing mRNA localization, Trends Cell Biol. 20 (2010) 380–390.
[51] S. Govindarajan, K. Nevo-Dinur, O. Amster-Choder, Compartmentalization and spatio-temporal organization of macromolecules in bacteria, FEMS Microbiol. Rev. 36 (2012) 1005–1022.
[52] E. Bertrand, P. Chartrand, M. Schaefer, S.M. Shenoy, R.H. Singer, R.M. Long, Localization of ASH1 mRNA particles in living yeast, Mol. Cell 2 (1998) 437–445.
[53] D. Fusco, N. Accornero, B. Lavoie, S.M. Shenoy, J.M. Blanchard, R.H. Singer, E. Bertrand, Single mRNA molecules demonstrate probabilistic movement in living mammalian cells, Curr. Biol. 13 (2003) 161–167.
[54] I. Golding, E.C. Cox, RNA dynamics in live *Escherichia coli* cells, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 11310–11315.
[55] C.C. Guet, L. Bruneaux, T.L. Min, D. Siegal-Gaskins, I. Figueroa, T. Emonet, P. Cluzel, Minimally invasive determination of mRNA concentration in single living bacteria, Nucleic Acids Res. 36 (2008) e73.
[56] T.T. Le, S. Harlepp, C.C. Guet, K. Dittmar, T. Emonet, T. Pan, P. Cluzel, Real-time RNA profiling within a single bacterium, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 9160–9164.
[57] K. Nevo-Dinur, S. Govindarajan, O. Amster-Choder, Subcellular localization of RNA and proteins in prokaryotes, Trends Genet. 28 (2012) 314–322.
[58] F. Jacob, J. Monod, Genetic regulatory mechanisms in the synthesis of proteins, J. Mol. Biol. 3 (1961) 318–356.
[59] M. Cohn, Contributions of studies on the beta-galactosidase of *Escherichia coli* to our understanding of enzyme synthesis, Bacteriol. Rev. 21 (1957) 140–168.
[60] M. Valencia-Burton, R.M. McCullough, C.R. Cantor, N.E. Broude, RNA visualization in live bacterial cells using fluorescent protein complementation, Nat. Methods 4 (2007) 421–427.
[61] M.W. Chen, V. Nagarajan, Chloramphenicol acetyltransferase, a cytoplasmic protein is incompatible for export from *Bacillus subtilis*, J. Bacteriol. 175 (1993) 5697–5700.
[62] K. Nevo-Dinur, A. Nussbaum-Shochat, S. Ben-Yehuda, O. Amster-Choder, Translation-independent localization of mRNA in *E. coli*, Science 331 (2011) 1081–1084.
[63] K. Schnetz, C. Toloczyki, B. Rak, Beta-glucoside (bgl) operon of *Escherichia coli* K-12: nucleotide sequence, genetic organization, and possible evolutionary relationship to regulatory components of two *Bacillus subtilis* genes, J. Bacteriol. 169 (1987) 2579–2590.
[64] I. Prasad, S. Schaefler, Regulation of the beta-glucoside system in *Escherchia coli* K-12, J. Bacteriol. 120 (1974) 638–650.
[65] O. Amster-Choder, The bgl sensory system: a transmembrane signaling pathway controlling transcriptional antitermination, Curr. Opin. Microbiol. 8 (2005) 127–134.
[66] J.K. Fisher, A. Bourniquel, G. Witz, B. Weiner, M. Prentiss, N. Kleckner, Four-dimensional imaging of *E. coli* nucleoid organization and dynamics in living cells, Cell 153 (2013) 882–895.
[67] M.D. Marger, M.H. Saier Jr., A major superfamily of transmembrane facilitators that catalyse uniport, symport and antiport, Trends Biochem. Sci. 18 (1993) 13–20.
[68] O. Yarchuk, N. Jacques, J. Guillerez, M. Dreyfus, Interdependence of translation, transcription and mRNA degradation in the lacZ gene, J. Mol. Biol. 226 (1992) 581–596.
[69] P.W. Postma, J.W. Lengeler, G.R. Jacobson, Phosphoenolpyruvate:carbohydrate phosphotransferase systems of bacteria, Microbiol. Rev. 57 (1993) 543–594.
[70] L. Lopian, A. Nussbaum-Shochat, K. O'Day-Kerstein, A. Wright, O. Amster-Choder, The BglF sensor recruits the BglG transcription regulator to the membrane and releases it on stimulation, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 7099–7104.
[71] L. Lopian, Y. Elisha, A. Nussbaum-Shochat, O. Amster-Choder, Spatial and temporal organization of the *E. coli* PTS components, EMBO J. 29 (2010) 3630–3645.
[72] V.T. dos Santos, A.W. Bisson-Filho, F.J. Gueiros-Filho, DivIVA-mediated polar localization of ComN, a posttranscriptional regulator of *Bacillus subtilis*, J. Bacteriol. 194 (2012) 3661–3669.
[73] M. Kaufenstein, M. van der Laan, P.L. Graumann, The three-layered DNA uptake machinery at the cell pole in competent *Bacillus subtilis* cells is a stable complex, J. Bacteriol. 193 (2011) 1633–1642.
[74] D.W. Reid, C.V. Nicchitta, Primary role for endoplasmic reticulum-bound ribosomes in cellular translation identified by ribosome profiling, J. Biol. Chem. 287 (2012) 5518–5527.
[75] Q. Chen, S. Jagannathan, D.W. Reid, T. Zheng, C.V. Nicchitta, Hierarchical regulation of mRNA partitioning between the cytoplasm and the endoplasmic reticulum of mammalian cells, Mol. Biol. Cell 22 (2011) 2646–2658.
[76] E. Bibi, Is there a twist in the *Escherichia coli* signal recognition particle pathway? Trends Biochem. Sci. 37 (2012) 1–6.
[77] G.D. Shockman, J.F. Barrett, Structure, function, and assembly of cell walls of gram-positive bacteria, Annu. Rev. Microbiol. 37 (1983) 501–527.
[78] M.J. Osborn, J.E. Gander, E. Parisi, J. Carson, Mechanism of assembly of the outer membrane of *Salmonella typhimurium*. Isolation and characterization of cytoplasmic and outer membrane, J. Biol. Chem. 247 (1972) 3962–3972.
[79] D.G. Thanassi, S.J. Hultgren, Multiple pathways allow protein secretion across the bacterial outer membrane, Curr. Opin. Cell Biol. 12 (2000) 420–430.
[80] D.J. du Plessis, N. Nouwen, A.J. Driessen, The Sec translocase, Biochim. Biophys. Acta 1808 (2011) 851–865.

[81] A.J. Driessen, N. Nouwen, Protein translocation across the bacterial cytoplasmic membrane, Annu. Rev. Biochem. 77 (2008) 643–667.
[82] P.A. Lee, D. Tullman-Ercek, G. Georgiou, The bacterial twin-arginine translocation pathway, Annu. Rev. Microbiol. 60 (2006) 373–395.
[83] R.E. Dalbey, P. Wang, A. Kuhn, Assembly of bacterial inner membrane proteins, Annu. Rev. Biochem. 80 (2011) 161–187.
[84] O. Schneewind, D.M. Missiakas, Protein secretion and surface display in Gram-positive bacteria, Philos. Trans. R. Soc. Lond. B Biol. Sci. 367 (2012) 1123–1139.
[85] J.E. Galan, H. Wolf-Watz, Protein delivery into eukaryotic cells by type III secretion machines, Nature 444 (2006) 567–573.
[86] M. Grynberg, A. Godzik, The signal for signaling, found, PLoS Pathog. 5 (2009) e1000398.
[87] A.D. Gazi, S.N. Charova, N.J. Panopoulos, M. Kokkinidis, Coiled-coils in type III secretion systems: structural flexibility, disorder and biological implications, Cell. Microbiol. 11 (2009) 719–729.
[88] G.I. Viboud, J.B. Bliska, *Yersinia* outer proteins: role in modulation of host cell signaling responses and pathogenesis, Annu. Rev. Microbiol. 59 (2005) 69–89.
[89] D.M. Anderson, O. Schneewind, *Yersinia enterocolitica* type III secretion: an mRNA signal that couples translation and secretion of YopQ, Mol. Microbiol. 31 (1999) 1139–1148.
[90] D.M. Anderson, O. Schneewind, A mRNA signal for the type III secretion of Yop proteins by *Yersinia enterocolitica*, Science 278 (1997) 1140–1143.
[91] K.S. Ramamurthi, O. Schneewind, *Yersinia enterocolitica* type III secretion: mutational analysis of the yopQ secretion signal, J. Bacteriol. 184 (2002) 3321–3328.
[92] K. Schesser, E. Frithz-Lindsten, H. Wolf-Watz, Delineation and mutational analysis of the *Yersinia pseudotuberculosis* YopE domains which mediate translocation across bacterial and eukaryotic cellular membranes, J. Bacteriol. 178 (1996) 7227–7233.
[93] M.P. Sory, A. Boland, I. Lambermont, G.R. Cornelis, Identification of the YopE and YopH domains required for secretion and internalization into the cytosol of macrophages, using the cyaA gene fusion approach, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 11998–12002.
[94] J. Trcek, G. Wilharm, C.A. Jacobi, J. Heesemann, *Yersinia enterocolitica* YopQ: strain-dependent cytosolic accumulation and post-translational secretion, Microbiology 148 (2002) 1457–1465.
[95] A. Holmstrom, R. Rosqvist, H. Wolf-Watz, A. Forsberg, Virulence plasmid-encoded YopK is essential for *Yersinia pseudotuberculosis* to cause systemic infection in mice, Infect. Immun. 63 (1995) 2269–2276.
[96] T. Minamino, K. Namba, Self-assembly and type III protein export of the bacterial flagellum, J. Mol. Microbiol. Biotechnol. 7 (2004) 5–17.
[97] K. Majander, L. Anton, J. Antikainen, H. Lang, M. Brummer, T.K. Korhonen, B. Westerlund-Wikstrom, Extracellular secretion of polypeptides using a modified *Escherichia coli* flagellar secretion apparatus, Nat. Biotechnol. 23 (2005) 475–481.
[98] G.S. Niemann, R.N. Brown, I.T. Mushamiri, N.T. Nguyen, R. Taiwo, A. Stufkens, R.D. Smith, J.N. Adkins, J.E. McDermott, F. Heffron, RNA type III secretion signals that require Hfq, J. Bacteriol. 195 (2013) 2119–2125.
[99] A. Bobrie, M. Colombo, G. Raposo, C. Thery, Exosome secretion: molecular mechanisms and roles in immune responses, Traffic 12 (2011) 1659–1668.
[100] B.L. Deatherage, B.T. Cookson, Membrane vesicle release in bacteria, eukaryotes, and archaea: a conserved yet underappreciated aspect of microbial life, Infect. Immun. 80 (2012) 1948–1957.
[101] S.I. Miller, M. Bader, T. Guina, Bacterial vesicle formation as a mechanism of protein transfer to animals, Cell 115 (2003) 2–3.
[102] S.N. Wai, B. Lindmark, T. Soderblom, A. Takade, M. Westermark, J. Oscarsson, J. Jass, A. Richter-Dahlfors, Y. Mizunoe, B.E. Uhlin, Vesicle-mediated export and assembly of pore-forming oligomers of the enterobacterial ClyA cytotoxin, Cell 115 (2003) 25–35.
[103] F. Jacob, D. Perrin, C. Sanchez, J. Monod, Operon: a group of genes with the expression coordinated by an operator, C. R. Hebd. Seances Acad. Sci. 250 (1960) 1727–1729.
[104] J. Prilusky, E. Bibi, Studying membrane proteins through the eyes of the genetic code revealed a strong uracil bias in their coding mRNAs, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 6662–6666.
[105] V. Van de Bor, I. Davis, mRNA localisation gets more complex, Curr. Opin. Cell Biol. 16 (2004) 300–307.
[106] R.S. Hamilton, I. Davis, RNA localization signals: deciphering the message with bioinformatics, Semin. Cell Dev. Biol. 18 (2007) 178–185.
[107] C.L. Woldringh, The role of co-transcriptional translation and protein translocation (transertion) in bacterial chromosome segregation, Mol. Microbiol. 45 (2002) 17–29.
[108] I. Fishov, V. Norris, Membrane heterogeneity created by transertion is a global regulator in bacteria, Curr. Opin. Microbiol. 15 (2012) 724–730.
[109] E.A. Libby, M. Roggiani, M. Goulian, Membrane protein expression triggers chromosomal locus repositioning in bacteria, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 7445–7450.
[110] I. Fishov, C.L. Woldringh, Visualization of membrane domains in *Escherichia coli*, Mol. Microbiol. 32 (1999) 1166–1172.
[111] P.J. Lewis, S.D. Thaker, J. Errington, Compartmentalization of transcription and translation in *Bacillus subtilis*, EMBO J. 19 (2000) 710–718.
[112] W. Wang, G.W. Li, C. Chen, X.S. Xie, X. Zhuang, Chromosome organization by a nucleoid-associated protein in live bacteria, Science 333 (2011) 1445–1449.
[113] S. Bakshi, A. Siryaporn, M. Goulian, J.C. Weisshaar, Superresolution imaging of ribosomes and RNA polymerase in live *Escherichia coli* cells, Mol. Microbiol. 85 (2012) 21–38.
[114] V. Khemici, L. Poljak, B.F. Luisi, A.J. Carpousis, The RNase E of *Escherichia coli* is a membrane-binding protein, Mol. Microbiol. 70 (2008) 799–813.
[115] G.G. Liou, W.N. Jane, S.N. Cohen, N.S. Lin, S. Lin-Chao, RNA degradosomes exist in vivo in *Escherichia coli* as multicomponent complexes associated with the cytoplasmic membrane via the N-terminal region of ribonuclease E, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 63–68.
[116] A. Taghbalout, L. Rothfield, RNaseE and RNA helicase B play central roles in the cytoskeletal organization of the RNA degradosome, J. Biol. Chem. 283 (2008) 13850–13855.
[117] M. Lehnik-Habrink, M. Schaffer, U. Mader, C. Diethmaier, C. Herzberg, J. Stulke, RNA processing in *Bacillus subtilis*: identification of targets of the essential RNase Y, Mol. Microbiol. 81 (2011) 1459–1473.
[118] A. Hunt, J.P. Rawlins, H.B. Thomaides, J. Errington, Functional analysis of 11 putative essential genes in *Bacillus subtilis*, Microbiology 152 (2006) 2895–2907.
[119] P. Montero Llopis, A.F. Jackson, O. Sliusarenko, I. Surovtsev, J. Heinritz, T. Emonet, C. Jacobs-Wagner, Spatial organization of the flow of genetic information in bacteria, Nature 466 (2010) 77–81.
[120] G. Storz, J. Vogel, K.M. Wassarman, Regulation by small RNAs in bacteria: expanding frontiers, Mol. Cell 43 (2011) 880–891.
[121] C.K. Vanderpool, S. Gottesman, Involvement of a novel transcriptional activator and small RNA in post-transcriptional regulation of the glucose phosphoenolpyruvate phosphotransferase system, Mol. Microbiol. 54 (2004) 1076–1089.
[122] K. Kimata, Y. Tanaka, T. Inada, H. Aiba, Expression of the glucose transporter gene, ptsG, is regulated at the mRNA degradation step in response to glycolytic flux in *Escherichia coli*, EMBO J. 20 (2001) 3587–3595.
[123] J.A. Opdyke, J.G. Kang, G. Storz, GadY, a small-RNA regulator of acid response genes in *Escherichia coli*, J. Bacteriol. 186 (2004) 6698–6705.
[124] T.A. Azam, S. Hiraga, A. Ishihama, Two types of localization of the DNA-binding proteins within the *Escherichia coli* nucleoid, Genes Cells 5 (2000) 613–626.
[125] E. Diestra, B. Cayrol, V. Arluison, C. Risco, Cellular electron microscopy imaging reveals the localization of the Hfq protein close to the bacterial membrane, PLoS One 4 (2009) e8301.
[126] M. Campos, C. Jacobs-Wagner, Cellular organization of the transfer of genetic information, Curr. Opin. Microbiol. 16 (2013) 171–176.
[127] J.A. Bernstein, A.B. Khodursky, P.H. Lin, S. Lin-Chao, S.N. Cohen, Global analysis of mRNA decay and abundance in *Escherichia coli* at single-gene resolution using two-color fluorescent DNA microarrays, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 9697–9702.